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IMPACT OF ASPARTAME CONSUMPTION ON NEUROTRANSMITTERS IN RAT BRAIN

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Abstract

Background: Aspartame (APM), a common artificial sweetener, has been used for diabetic subjects and body weight control for a long time. The goal of the present study was to evaluate the impact of APM consumption on neurotransmitters and oxidative stress in rat's brain.

Materials and Methods: Four groups of male Wistar albino rats was used: Group1, control (Rats fed on normal diet). Group 2: Rats were received aspartame (50 mg/kg b w). Group3: Rats were received aspartame (75 mg/kg b w). Group 4: Rats were received aspartame (125 mg/kg b w). Five rats were decapitated after 10, 20, 30 and 40 days from start of experiment. Blood and brain tissue were collected for biochemical analysis. Biochemical analysis of brain tissue includes neurotransmitters (Acetylcholine, epinephrine, norepinephrine, γ-aminobutyric acid and serotonin). Serum for determination of lipid peroxidation (MDA), reduced glutathione and superoxide dismutase (SOD).

Results: the data obtained showed that antioxidant activities (SOD and GSH) were reduced significantly ($p<0.001$) while malondialdhyde (MDA) level was increased compared with control. Brain neurotransmitters levels (serotonin, GABA and dopamine) were reduced significantly compared with control ($p<0.001$, <0.01) after consumption of APM. However, the level of acetylcholine and norepinephrine increased in rats fed AMP compared with control ($p<0.001$). The effect of APM is dose dependent.

Conclusion: Consumption of APM for a long time increased oxidative stress in brain tissue and disruption in neurotransmitters that affect physiological functions.

Key words: Aspartame, neurotransmitters, oxidative Stress, rats.

Introduction

Aspartame (APM) (L-aspartyl-L-phenylalanine methyl ester) is a dipeptide, and is considered as one of the common artificial sweeteners used by FDA (Weihrauch and Diehl, 2004). This sweetener gained a lot of attention due to its low calories while retaining sweet taste, low price and potential health effects, such as reduction in body weight and for the management of diabetes (Olivier et al., 2015). APM was increasingly introduced in many food products as diet sodas, cereals, sugar-free desserts, dairy products and pharmaceuticals formulations (Gardner et al., 2012).After ingestion, APM is easily absorbed from the intestinal mucosal cell and hydrolyzed to phenylalanine, methanol and aspartic acid (Ranney et al., 1976). Consequently , high doses of aspartame consumption increased the levels of this intermediates in the circulation (Stegink, 1987).Previous study suggested that high doses of aspartame or its individual metabolites particularly methanol exert toxic effect on the brain (Kruse, 1992). It was reported that methanol is primarily oxidized to formaldehyde and then into formate, that is involved in methanol toxicity in human. This is accompanied by production of superoxide anion and hydrogen peroxide (Lee et al., 1994, Eells et al., 2000, Parthasarathy et al., 2006).There are a few data showed the effect of aspartame consumption on specific neurological conditions such as headache, insomnia and seizures (Johns, 1986), alterations in regional concentrations of catecholamine which is accompanied with behavioral disturbances (Coulombe and Sharma, 1986, Humphries et al., 2008). The rational of this study was to investigate whether long-term uses of aspartame can affect oxidative stress and brain neurotransmitters in albino rats

Material and Methods

Chemicals and Kits

Aspartame (L aspartyl- L-phenylalanine methyl ester) was purchased from Noga-biochem, Shandong–china. Biochemical parameter Kits for the determination of brain tissue was obtained from Bio-diagnostic Company, Jeddah, Saudi Arabia. All tools that were used in the experiment were from Shafi'i Foundation for Medical Equipment, Jeddah, Chemicals that used to determination of the blood was obtained from sigma-Aldrich (Gillinham, UK).

Experimental animals

One hundred and sixty male *albino* rats, age 4 weeks and weighting 120-150 g, were obtained from the Animal House of King Fahd Medical Research Center (KFMRC), King Abdulaziz University (Jeddah, Saudi Arabia). Rats were placed in stainless cages (5 rats/cage). Rats were fed standard diet obtained from Saudi Company for oils, Jeddah. All animals were allowed one week to acclimatize in animal housing conditions before being used for the study. Experiments were conducted according to the Ethical Committee of King Abdulaziz University (Jeddah, Saudi Arabia).
Animals were randomly divided into four groups (each 40 rats). Group I, served as control group. Rats in groups II-IV were fed aspartame orally at a dose of 50, 75 and 125 mg/kg BW/day respectively for 40 days (Labra-ruiz et al., 2007).

Blood and brain tissue collection

Blood and brain samples were collected after 10, 20, 30 and 40 days from the start of the experiment. At the end of the experiment, rats were anesthetized with thiopental and whole blood was collected and brain tissue was quickly removed and cleaned. Blood samples were centrifuged at 4°Cat 3000 rpm and serum was kept in Eppendorf tubes, brain was kept at -80°Cfor further analysis.

Biochemical analysis

Assay of acetylcholine

Acetylcholine (ACh) was determined according to Vizi et al. (1985) by Enzyme-linked Immuno-Sorbent Assay (ELISA) using Choline/Acetylcholine Assay Kit (Biosource International, USA) .The colorimetric assay of GABA was performed in presence of glutamate decarboxylase (YANG Sheng-Yuan, 2006).The serotonin level in the brain was determined by fluorescence method (Anderson et al., 1992). A fluorometric method was developed for the determination of norepinephrine and epinephrine (Fiorica and Moses, 1971). Reduced glutathione (GSH) was determined according to Baker et al. (1990). SOD was determined by the method of Beauchamp and Fridovich (1971). Plasma MDA level was measured by the modified method of Satoh (Kei, 1978).

Statistical analysis

Statistical analysis was done by SPSS version 20, SPSS Inc., Chicago, IL, USA) was used for data analysis. A probability (P) <0.05 was considered significant.

Results

Data presented in Table1 showed that, after 40 days of aspartame feeding, there was a significant increase in body weight at 50 or 75 mg/kg/body ($p<0.01$ and <0.001) respectively compared with control, whereas non-significant changes in body weight at 125 mg/kg. The increase in body weight was correlated with time as compared with control group ($p<0.001$).

The levels of serotonin, GABA and dopamine were reduced in the brain tissue homogenate in rats given 50, 75 and 125 mg/kg ($p<0.05$, $P<0.001$ and $p<0.001$) respectively compared to control. In contrast, the level of Ach at doses 50, 75, and 125 mg/kg was significantly higher than control group ($P <0.01$, $P<0.001$ and $p<0.001$) respectively, as well as of norepinephrine at same doses ($p<0.05$). The level of epinephrine in treated rats were increased only at doses 50 and 75mg/kg AMP compared with control group ($P <0.05$, $P<0.001$ and $p<0.001$). The GSH levels of brain homogenate in the aspartame-treated rats were shown in Table 3. The results showed that there were non-significant changes at days 10, 20 and 40 in the studied groups. Interestingly, at day 30, the glutathione levels in rat fed on aspartame 50 and 125 mg/kg/day were significantly lower than control ($P<0.001$ and $p<0.001$, $p<0.05$) respectively. The activities of SOD in different studied groups at different durations are shown in Table 4. It was found that at days 30 and 40, there were no significant changes of brain SOD compared with control. However, at day 10, rat fed on aspartame 75 mg/kg/day or 125 mg/kg/day were significantly higher than control ($P<0.01$ and $p<0.001$respectively). At day 20, rat fed on aspartame 50 mg/kg/day and

rat fed on aspartame 75 mg/kg/day were significantly higher than control (P =0.024 and P =0.023, respectively). Table 5 showed that at days 20 and 30, there were non-significant changes in brain homogenate levels of MDA in different studied groups. However, at days 10, the concentrations of 75 and 125 mg/kg/day, the brain MDA levels were significantly higher than the controls (P =0.025 and P =0.006, respectively), and also at day 40, the brain MDA levels with concentration of 50 and 125 mg/kg/day were significantly higher than the controls (P =0.049 and P =0.025, respectively).

Table1: Initial and final body weight (g) of different groups at different times (mean± SD)

Experimental groups / Variables	Control	50 mg/kg	75 mg/kg	125 mg/kg
Body weight (initial) (g)	142.9±20.16	123.3±24.8	139.2±27.78	153.3±14.92
Body weight (final) (g)	326.7±25.16	370±20	363.3±11.54	310±30

Results were expressed as mean ± SD ($p<0.05$), analysis of variance for multiple comparison by Dennett's test. [a]: Significance vs. control

Table 2: The levels of neurotransmitters in brain tissue in all studied groups (mean± SD)

Groups	Control N=10	50 mg aspartame N=10	75 mg aspartame N=10	125 mg Aspartame N=10
Serotonin (µg /g)	90±19	33±8 [a]	20±2.5[a]	24±3.5[a]
GABA (µg /g)	42±3	66±6 [a]	53±9[a]	41±5[a]
Acetylcholine (µg/g)	11±1.5	23±1.3[a]	26±11[a]	39±7.5[a]
Norepinephrine (ng /g)	33±7	53±13 [a]	54±19[a]	60±16[a]
Epinephrine (ng /g)	18.1±2.5	90±13 [a]	109±19[a]	93±15[a]

Results were expressed as mean ± SD ($p<0.05$), analysis of variance for multiple comparison by Dennett's test. [a]: Significance vs. control. N: number of rats

Table 3: Effect of AMP on brain Glutathione (mg/dl) in different studied groups at different days.

Time / Groups	10 days N=10	20 days N=10	30 days N=10	40 days N=10
Control	2.61±1.27	3.44±0.50	3.31±0.13	3.19±0.29
APM (50 mg/kg/day)	3.58±1.30	3.24±0.34	2.26±0.52[a]	3.36±0,87
APM (75 mg/kg/day)	2.84±0.96	2.61±9.12	2.66±0.49[a]	3.57±0.96
APM (125 mg/kg/day)	3.06±1.72	2.88±0.76	2.28±0.13[a]	3.45±0.80

Data are expressed as mean ± standard deviation. [a] Denotes that the data are significantly different from the control group at P< 0.05. Analysis was made using ONEWAY ANOVA test (LSD). N: number of rats

Table 4: Brain levels of SOD (U/L) in different studied groups at different days.

Groups	10 days N=10	20 days N=10	30 days N=10	40 days N=10
Control	2216.04±149.85	1940.19±38.72	2331.94±226.23	2283.26±550.09
APM (50 mg/kg/day)	2515.07±104.62	2714.42±322.02[a,]	2496.52±12.04	2777.00±286.97[a,]
APM (75 mg/kg/day)	2946.22±383.19[a, b]	2719.05±261.40[a,]	2515.07±182.72	2603.15±74.35
APM (125 mg/kg/day)	2929.99±281.73[a, b]	2338.90±537.96	2306.44±104.39	2399.17±163.53

Data are expressed as mean ± standard deviation. [a] Denotes that the data are significantly different from the control group at P< 0.05. [b] Denotes that the data are significantly different from APM (50mg/kg) at P < 0.05. Analysis was made using ONEWAY ANOVA test (LSD). N: number of rats

Table 5: Brain levels of MDA (nm/dl) in different studied groups at different days

Groups	10 days N=10	20 days N=10	30 days N=10	40 days N=10
Control	20.30±7.63	28.52±5.74	26.39±4.85	25.88±12.53
APM (50 mg/kg/day)	20.30±7.63	34.86±1.54	35.07±12.42	38.92±5.18[a]
APM (75 mg/kg/day)	37.24±8.87[a, b]	41.16±4.56	32.06±5.88	33.34±1.98
APM (125 mg/kg/day)	43.14±5.54[a, b]	40.16±3.44	32.06±5.88	41.42±1.41[a]

Data are expressed as mean ± standard deviation. [a]Denotes that the data are significantly different from the control group at P< 0.05. Analysis was made using ONEWAY ANOVA test (LSD). N: number of rats

Discussion

The goal of the present study was to evaluate the impact of long-term use of APM on neurotransmitter levels and oxidative stress in the brain of albino rats. Neurotransmitters are chemical mediators released in response to stimuli that change activity in neurons, when chemical molecules pass over a synapse they attach to special receiving areas on the next neuron and exert its action (Sudhof, 2013). The effects of APM on neurotransmitters ACh, serotonin, GABA, epinephrine (E) and norepinephrine (NE) were investigated. Results obtained showed a significant elevation in brain neurotransmitters ACh, norepinephrine, epinephrine and serotonin (p value<0.01, 0.001, 0.05, 0.001) respectively, compared with control. The AMP composed of 50% phenylalanine, 40% aspartic acid and methanol (10%). Phenylalanine is the precursors of catecholamines, therefore, NE and E were elevated on long term utilization. On the same time, tryptophan will not be adequately carried across the BBB and serotonin production will be compromised in APM administered. The NE and E levels in brain increased significantly.

Coulombe and Sharma (1986) analyzed different regions of brain for catecholamine and serotonin neurotransmitters and their major metabolites. They reported that animals given single doses of APM increased adrenergic chemical mediators, which were not apparent after repeated doses. However, a decreased serotonin and its metabolite (5-hydroxyindoleacetate) was found in several regions.

Oxidative damage lead to endothelial abnormality by changing NO release in the circulation (Pandey and Rizvi, 2010). Based on our results, APM treatment induced lipid peroxidation and increased levels of MDA that considered as a marker of oxidative stress(Humphries et al., 2008). In this study rats injected with APM showed a significant elevation in the level of MDA as compared with control. MDA levels increased by increasing the dose and duration of AMP injection ($P<0.001$). The Antioxidants potential to remove free radicals act by different mechanism as prevention, scavenger and repair. Preventive antioxidants attributed to stop the formation of reactive oxygen species (ROS). These include SOD that catalysis the dismutation of superoxide to H_2O_2 and catalase that breaks it down to water. Interception of free radicals is mainly by radical scavenging, while at the secondary level scavenging of peroxyl radicals are affected. The effectors include various antioxidants like vitamins C and E, glutathione, other thiol compounds, carotenoids, flavonoids, etc. At the repair and reconstitution level, mainly repair enzymes are involved (Sies 1996). The elevation of MDA is accompanied with reduction in GSH and superoxide dismutase ($P<0.001$ and < 0.01, respectively).

The reduction in glutathione level observed in our study may be attributed to methanol release from aspartame, this is due to methanol metabolism depends upon glutathione (Parthasarathy et al., 2006). The reduction of reduced glutathione, elevation of oxidized GSSG and decrease of the reduced/oxidized ratio increase cell sensitivity to oxidative stress (Shelly et al., 2009). This alteration may be due to the production of free radicals by methanol intermediate of APM. Reduced form of glutathione prevent the cellular content from attack to the toxic effects of lipid peroxidation (Shelly et al., 2009). Normally, GSH can bind with superoxide and hydroxyl radical (Atasayar et al., 2009). The results obtained may provide evidence that APM intake enhances generation of oxidative stress in rat by altering the glutathione ratio status.

In conclusion, long term APM consumption increased level of lipid peroxidation and reduction in antioxidant activities compared with control. In addition, it caused disruption in brain concentrations of catechol-amines. However, APM should be used under control and physician advice.

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Declaration: Authors declare that this research presents no conflict of interests.

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